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Published in:
European Journal of Medical Genetics

DOI (link to publication from Publisher):
10.1016/j.ejmg.2021.104183

Publication date:
2021

Document Version
Publisher's PDF, also known as Version of record

European Journal of Medical Genetics 64 (2021) 104183

Contents lists available at ScienceDirect

European Journal of Medical Genetics

journal homepage: www.elsevier.com/locate/ejmg

Original article

Clinical genetic diagnostics in Danish autosomal dominant polycystic kidney disease patients reveal possible founder variants

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ARTICLE INFO

Keywords:
ADPKD
Founder variants
Recurrent variants
Targeted NGS
Variant classification

ABSTRACT

Background: Autosomal dominant polycystic kidney disease (ADPKD) is the most common heritable kidney disease. ADPKD leads to cysts, kidney enlargement and end-stage renal disease. ADPKD is mainly caused by variants in *PKD1* and *PKD2*, with truncating *PKD1* variants causing the most severe phenotype. This study aimed to characterize variants in Danish patients referred for screening of genes related to cystic kidney disease.
Methods: 147 families were analysed for variants in *PKD1*, *PKD2* and *GANAB* using next generation sequencing and multiplex ligation-dependent probe amplification. If a variant was identified, relatives were analysed for the specific variant using Sanger sequencing.
Results: A pathogenic or possibly pathogenic variant was identified in 87% (103/118) of patients suspected to suffer from ADPKD, according to the requisition form. In total, 112 pathogenic or possibly pathogenic variants were observed, of which 94 were unique; 74 (79%) in *PKD1* and 20 (21%) in *PKD2,* while 41 variants were novel. No variants in *GANAB* were observed. Ten recurrent variants were observed in 26 (26%) families. These were either *PKD2* variants (N = 6) or non-truncating *PKD1* variants (N = 4). Five of these were likely founder variants.
Conclusions: The distribution of pathogenic or possibly pathogenic variants in the Danish ADPKD population is similar to that in other populations, except that recurrent truncating *PKD1* variants appear to be rare, i.e. founder variants tend to be variant types associated with a mild phenotype. Patients with a mild phenotype may remain undiagnosed, consequently the frequency of founder variants and prevalence of ADPKD may be underestimated.

1. Introduction

Autosomal dominant polycystic kidney disease (ADPKD) is the most common monogenic kidney disease (Torres and Harris, 2009). The disease leads to cyst formation and growth, massive kidney enlargement and, ultimately, end-stage renal disease (Rossetti et al., 2007).

Most cases of ADPKD appear to be caused by heterozygosity for a pathogenic variant in *PKD1* or *PKD2*: according to the literature, variants in *PKD1* are identified in ~85% of ADPKD families with a known pathogenic variant, while variants in *PKD2* are identified in ~15% of these families (Rossetti et al., 2007; Harris and Torres, 2014; Cornec-Le Gall et al., 2014; Paul and Vanden Heuvel, 2014). Within each of these genes, several thousands of unique variants are reported to be pathogenic or likely pathogenic (Eggermann et al., 2006). Recently, heterozygosity for variants in *GANAB* and *DNAJB11* were reported to cause ADPKD in 0.3% and 0.1% of families, respectively (Porath et al., 2016; Cornec-Le Gall et al., 2018). Inter- and intra-familiar variability of the phenotype is observed for all types of variants, however, patients with truncating variants in *PKD1* (large rearrangements, frameshifts, indels and variants affecting canonical splice sites) tend to have the most severe phenotype (Hwang et al., 2016).

Despite the large number of ADPKD patients undergoing genetic testing, it is often difficult to determine whether a variant in *PKD1* or *PKD2* is pathogenic, mostly because many variants are missense

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https://doi.org/10.1016/j.ejmg.2021.104183
Received 10 September 2020; Received in revised form 4 January 2021; Accepted 20 February 2021
Available online 24 February 2021

variants, many are only identified in one family and some patients carry multiple variants (Carrera et al., 2016). Compiling findings in multiple studies of ADPKD families can be helpful in the classification of variants.

In order to further characterize variants associated with ADPKD, we report the distribution of pathogenic or possibly pathogenic variants in Danish patients referred for screening of *PKD1*, *PKD2,* and *GANAB* during a five-year period. In addition, we report the observed novel variants associated with ADPKD and the occurrence of recurrent variants.

2. Material and methods

2.1. Patients

This is a retrospective study including samples (N = 147) sent to the Department of Clinical Genetics, Aarhus University Hospital (AUH), Denmark, for screening of genes associated with cystic kidney disease from April 2014 to April 2019. This public laboratory was the first to offer the analysis in Denmark; therefore, most Danish patients analysed in this 5-year period were included in the present study. Screening was requested either from a department of clinical genetics or from a department of nephrology.

Eighty-one patients (55%) were females. The median age at the time of laboratory analysis was 47 years (range: 0–76 years); 135 patients (92%) were 18 years or older at the time of analysis.

Data including personal identifiers, the clinical indication for performing the analysis as stated on the requisition form, type of tissue, method(s) and results were retrieved from the laboratory database of the Department of Clinical Genetics, AUH. In Denmark, quality control projects like the present, are exempted from approval by institutional review boards. As this study took place in a department of clinical genetics, we did not have access to systematic and valid information about the phenotype, e.g. the severity of the disease. However, we did have access to pedigrees constructed at the departments of clinical genetics during the genetic work-up.

We scrutinised the clinical information given on the requisition form. If it said that the patient was suspected of ADPKD or that the patient had cystic kidneys, we classified the patient as suspected to suffer from ADPKD. 118 patients fulfilled one of these criteria. For 27 patients there were clinical information on the requisition form, however neither ADPKD nor cystic kidneys were stated. For 2 patients no information regarding phenotype was given.

3. Molecular analysis

3.1. DNA extraction

DNA had been extracted from peripheral blood, tissue sampes and amnion cells.

3.2. Sequencing

The genes *PKD1* (NM_001009944.2), *PKD2* (NM_000297.3) and *GANAB* (NM_198335.5) had been sequenced using a next generation sequencing (NGS) panel targeting exons. 1 μg DNA was used for construction of a library for Illumina paired-end sequencing using the KAPA HTP Library Preparation Kit according to the manufacturer's instructions (KAPA Biosystems Inc., Wilmington, MA, USA). The libraries were enriched for regions of interest using a customized targeting probe set (SeqCap EZ Choice, Roche Nimblegen, Inc., Madison, WI, USA) and sequenced on a MiSeq or NextSeq Sequencer (Illumina, San Diego, CA, USA). The sequencing reads were aligned to the human genome (GRCh37), and variants were called in coding exons and flanking regions ± 10 bp, using Biomedical Genomics Workbench v.2 (CLC bio-Qiagen, Aarhus, Denmark). All regions not covered 30x were sequenced using direct Sanger sequencing; primer sequences and other PCR details are available upon request.

Relatives had been analysed for the family-specific variant(s) by direct Sanger sequencing using BigDye® Terminator v1.1 Cycle Sequencing Kit, as described by the manufacturer (Applied Biosystems, Life Technology) and analysed using ABI 3500xl Genetic Analyzer (Applied Biosystems, CA, USA). Further details are available upon request.

3.3. Multiplex ligation-dependent probe amplification assay

All samples in which no pathogenic or possibly pathogenic variant (as defined below), was detected by sequencing, had been analysed for deletion/duplication of one or more exons by multiplex ligation-dependent probe amplification (MLPA) using probe sets P351 and P352 following the manufacturer's instructions (MRC-Holland, Amsterdam, Holland).

3.4. Variant filtration and classification

Variants had been called in RefSeq for each gene, and the following variants had been excluded: synonymous variants except for those located ±2 bp of exon-intron borders; variants registered in a public database (ExAC, gnomAD, and/or whole-exome sequencing of 2000 Danish individuals (Lohmueller et al., 2013)) with a minor allele frequency (MAF) > 5% and variants detected in >10% of the patients registered in the in-house frequency database of the laboratory.

In the present study, the variants, that had been reported by the clinical laboratory as clinically relevant were classified as follows: Variants were deemed to be known if listed in the Autosomal Dominant Polycystic Kidney Disease: Mutation Database (PKDB) (Eggermann et al., 2006) and/or published in the Human Genome Mutation Database (HGMD, Qiagen) (Stenson et al., 2003). Novel variants were classified using VarSome (Kopanos et al., 2019) exclusively. The software classifies variants according to the American College of Medical Genetics & Genomics (ACMG) guidelines (Richards et al., 2015) developed for interpretation of sequence variants. Known variants were further classified based on information in the PKDB. Both novel and known variants were classified into two groups: pathogenic (P) or possibly pathogenic (PP), (Table 1). Only variants classified as pathogenic and definitely pathogenic by ACMG and PKDB, respectively, were classified as P. Among the remaining variants those that were either classified as pathogenic, likely pathogenic or variant of uncertain significance by ACMG, and/or definitely pathogenic, highly likely pathogenic, likely pathogenic, likely hypomorphic or variant of uncertain significance by PKDB were classified as PP. Using this algorithm, the group of variants classified as PP is quite heterogenous.

Segregation analysis was possible in 17 families with a PP variant; in two of these families, more than one informative meiosis was available. Included were only family members diagnosed with multiple bilateral kidney cysts and family members who were unaffected at the age of 40 years or above. For phenotypic classification of family members, we used clinical data from the requisition forms and pedigrees drawn at departments of clinical genetics.

Recurrent variants were accessed in the HGMD and the Danish Cystic Kidney Disease Registry developed by us, at the Department of Clinical Genetics, AUH, (Danish Data Protection Agency reference number: 1-16-02-364-13). This registry holds data on the phenotype and genotype of 97 families in which a minimum of one family member suffers from a cystic kidney disease and has received counselling at the Department of Clinical Genetics, AUH, or Aalborg University Hospital, and/or had *PKD1* and *PKD2* analysed prior to April 2014. The analysis had been performed by commercial laboratories in Europe at the request of Department of Clinical Genetics, AUH.

The description at protein level in all tables is given without any experimental evidence.

Table 1
Classification of variants.

PKDB	ACMG				
	Pathogenic	Likely pathogenic	Variant of uncertain significance	Likely benign	Benign
Definitely pathogenic	P[a]	PP	–	–	–
Highly likely pathogenic	–[b]	PP	PP	–	–
Likely pathogenic	–	PP	PP	–	PP
Likely hypomorphic	–	–	PP	–	PP
Variant of uncertain significance	–	–	–	–	PP
Not available	PP[c]	PP	PP	–	–

[a] P: Pathogenic.
[b] Combination not observed.
[c] PP: Possibly pathogenic.

3.5. Statistics

Differences in the distribution of variants in *PKD2* and non-truncating variants in *PKD1* (in-frame indels, alternative splicing and missense variants) in this study compared to other studies, were evaluated using Fisher's exact test ($p<0.05$).

4. Results

During a five-year period, 147 patients were screened for pathogenic variants in *PKD1*, *PKD2* and *GANAB*. Among 118 patients suspected to have ADPKD, a P or PP variant was identified in 103, producing a diagnostic yield of 87%. No P or PP variants were identified in the 29 patients with cystic kidneys, for whom a specific suspicion of ADPKD was not indicated on the requisition form.

In total, 112 P or PP variants were identified of which 94% (105/112) were identified using NGS and 6% (7/112) using MLPA. A total of 94 unique P or PP variants were observed: 74 in *PKD1* and 20 in *PKD2*. No variants were observed in *GANAB*. The distribution of P and PP variants according to gene, is given in Table 2. For detailed information of P and PP variants, see Supplementary Table 1 and Supplementary Table 2. All variants can be found in PKDB.

The majority of variants were missense (37%), followed by nonsense variants (27%), frameshift variants (18%), large rearrangements (7%), variants affecting canonical splice sites (6%), in-frame insertions/deletions of >5 amino acids (3%) and variants causing alternative splicing (1%), (Table 3).

For the 103 patients in whom P or PP variants were identified, pedigrees were assessed. None of these patients appeared to be related. In 63 patients, one variant in *PKD1* was identified, five patients had two variants in *PKD1,* 31 patients had one variant in *PKD2*, one patient had two variants in *PKD2* and three patients had one variant in *PKD1* and one variant in *PKD2* (Supplementary Table 3). In five of the nine patients with two variants, one variant was classified as P and the other as PP. Both variants in the remaining four patients were classified as PP. For one variant, segregation analysis was informative: One patient carried two PP variants in *PKD1*: c.4562G > T and c.9829C > T. However, segregation analysis indicated that c.9829C > T alone is insufficient to cause ADPKD as it was identified in an unaffected relative. According to the PKDB, the variant c.9829C > T is classified as likely hypomorphic and might contribute to disease when observed in combination with other variants.

Ten recurrent variants were observed in a minimum of 2 and up to 7 families. A total of 27 families harboured a recurrent variant. In one of these families, two recurrent variants were observed. Recurrent variants were identified both in *PKD1* (N = 4) and *PKD2* (N = 6). Eight of these variants were known, and two were novel, (Table 4). In the family harbouring two recurrent variants, one of these was c.8293C > T in *PKD1*. The frequency of this variant in the background population is 0.8565% according to GnomAD, and the variant is classified as benign according to VarSome and as likely hypomorphic according to the PKDB and the literature (Rossetti et al., 2009).

Table 2
Distribution of known and novel variants in *PKD1* and *PKD2*, by classification.

Gene	Classification	Known variants (N = 53)	Novel variants (N = 41)	Total distribution of variants (N = 94)
PKD1	Pathogenic	53% (20/38)	47% (18/38)	40% (38/94)
	Possibly pathogenic	53% (19/36)	47% (17/36)	38% (36/94)
PKD2	Pathogenic	62% (8/13)	% (5/13)	14% (13/94)
	Possibly pathogenic	86% (6/7)	14% (1/7)	7% (7/94)

5. Discussion

This study included 147 patients, of whom 118 were specifically suspected to suffer from ADPKD according to information given by the doctor requesting the gene analysis. A pathogenic or possibly pathogenic variant was identified in 103 of these 118 patients, whereas no such variants were observed among 29 patients for whom analysis of *PKD1, PKD2,* and *GANAB* was requested without the requisition form indicating a suspicion of ADPKD. Hence, the diagnostic yield was 70% (103/147) among all patients, and 87% (103/118) among patients specifically indicated to be suspected of ADPKD. The diagnostic yield was 80% in an Italian cohort in which 21.8% of the patients had an uncertain clinical diagnosis (Carrera et al., 2016). Yields in other studies of Caucasians range from 89.1 to 92.4% (Rossetti et al., 2007; Hwang et al., 2016; Audrezet et al., 2012; Heyer et al., 2016). Barring one (Hwang et al., 2016), these studies included patients diagnosed using Pei's criteria (Ravine et al., 1994). The yield observed in the present study is relatively high taking into account that in Denmark patients with a classic ADPKD phenotype and a family history are mainly diagnosed using Pei's criteria, only, unless prenatal diagnostics or predictive testing is requested (Autosomal dominant polycy, 2012), which should cause an increase in the fraction of patients with a mild or otherwise unusual phenotype among those analysed. Consistently, a non-significant ($p = 0.7215$ and $p = 0.1452$, respectively) but higher frequency of *PKD2* (21%) and non-truncating *PKD1* variants (36%) was identified among the Danish patients (Table 3) than in similar studies from other countries (Rossetti et al., 2007; Hwang et al., 2016; Audrezet et al., 2012; Heyer et al., 2016), in which the averages were 18% and 27%, respectively.

Identifying the cause of ADPKD is hampered by the fact that many cases are caused by missense variants that are difficult to classify beyond variant of uncertain significance (VUS). Furthermore, since most of these variants are unique to one family, it is difficult to obtain information that indicates association. As observed in Supplementary Table 2, several variants have been classified differently using VarSome and PKDB. While currently it is not known which classification method most correctly classifies ADPKD variants, aggregated data from multiple studies should improve variant classification. Furthermore, our classification system is strong with regard to reproducibility in that we used

Table 3
Distribution of pathogenic and possibly pathogenic variants in PKD1 and PKD2, by variant type.

	PKD1			*PKD2*			Total
Type of variant	Pathogenic (N = 38)	Possibly pathogenic (N = 36)	Total (N = 74)	Pathogenic (N = 13)	Possibly pathogenic (N = 7)	Total (N = 20)	(N = 94)
Large rearrangement[a]	7% (5/74)	1% (1/74)	8% (6/74)	5% (1/20)	0% (0/20)	5% (1/20)	7% (7/94)
Nonsense	27% (20/74)	1% (1/74)	28% (21/74)	20% (4/20)	0% (0/20)	20% (4/20)	27% (25/94)
Frame shift	15% (11/74)	3% (2/74)	18% (13/74)	20% (4/20)	0% (0/20)	20% (4/20)	18% (17/94)
Canonical splice site	3% (2/74)	0% (0/74)	3% (2/74)	20% (4/20)	0% (0/20)	20% (4/20)	6% (6/94)
In-frame indel >5 amino acids[b]	0% (0/74)	4% (3/74)	4% (3/74)	0% (0/20)	0% (0/20)	0% (0/20)	3% (3/94)
Atypical splicing	0% (0/74)	1% (1/74)	1% (1/74)	0% (0/20)	0% (0/20)	0% (0/20)	1% (1/94)
Missense	0% (0/74)	38% (28/74)	38% (28/74)	0% (0/20)	35% (7/20)	35% (7/20)	37% (35/94)

[a] Large rearrangements: deletion or duplication of one or more exons detected by multiplex ligation-dependent probe amplification.
[b] Indels: Insertions or deletions.

Table 4
Recurrent variants in PKD1 and PKD2.

Gene[a]	DNA change	Protein change	Number of families[b]	Number of meioses[d]	PKDB Classification[e]	GnomAD frequence (%)[f]	At CpG[g]	HGMD references[h]
PKD1	c.6643C > T	p. Arg2215Trp	2 + 0 = 2	0	LP	0.001893	Y	Cornec-Le Gall (2013) *J Am Soc Nephrol* 24: 1006; Kim (2019) *Sci Rep* 9: 16,952
PKD1	c.7115C > G	p. Ser2372Cys	2 + 0 = 2	1	LP	0	N	Audrézet (2012) *Hum Mutat* 33, 1239: Cornec-Le Gall (2013) *J Am Soc Nephrol* 24: 1006
PKD1	c.8293C > T	p. Arg2765Cys	2[c]+1 = 3	0	LH	0.8565	Y	Rossetti et al. (2009) *Kidney Int* 75, 848; Gonzalez-Paredes (2014) *Gene* 546: 243
PKD1	c.11249G > A	p. Arg3750Glu	2 + 0 = 2	NA	HLP	0	N	Hoefele (2010) *Nephrol Dial Transplant* 26, 2181; Cornec-Le Gall (2013) *J Am Soc Nephrol* 24: 1006; Carrera et al. (2016) *Sci Rep* 6: 30, 850
PKD2	c.1521G > A	p.Trp 507*	2 + 0 = 2	0	NA	0	Y	Robinson (2012) *BMC Nephrol* 13, 79
PKD2	c.(2392_2664)_(2861_?)del	p.?	2 + 1 = 3	0	NA	NA	N	NA
PKD2	c.2119-2 A > G	p.?	2 + 4 = 6	0	NA	0	N	NA
PKD2	c.2241-2 A > G	p.?	7[c]+2 = 9	1	DP	0	N	Veldhuisen (1997) *Am J Hum Genet* 61, 547
PKD2	c.2600 T > C	p.Leu867Pro	4 + 4 = 8	5	NA	0	N	Robinson (2012) *BMC Nephrol* 13, 79
PKD2	c.2614C > T	p.Arg 872*	3 + 2 = 5	1	DP	0.0008818	Y	Neumann (2013) *Nephrol Dial Transplant* 28, 1472

[a] *PKD1* (NM_001009944.2) and *PKD2* (NM_000297.3).
[b] Number of families in the dataset + number of families in the Danish Cystic Kidney Disease Registry.
[c] One family harbours both variants.
[d] Number of meioses in which the variant co-segregated with the phenotype for all families harbouring the variant.
[e] LP; Likely pathogenic; LH: Likely hypomorphic; HLP: Highly likely pathogenic; NA: Not available; DP: Definitely pathogenic.
[f] NA: Not available. [g]Variant located at CpG site; Y: Yes; N: No.
[g] NA: Not available. [g]Variant located at CpG site; Y: Yes; N: No.
[h] NA: Not available. [g]Variant located at CpG site; Y: Yes; N: No.

standard software (VarSome), rather than inventing our own scoring system.

In the present study, identical variants were detected in several families. Due to the unique civil registration number assigned to all people living in Denmark, we could ensure that these families were not closely related. Furthermore, some of the families had been subjected to genetic work-up including drawing of pedigrees. In total, 26% of the families had a recurrent variant (Table 4), which is similar to the 20–30% reported in other studies in Caucasians (Rossetti et al., 2007; Audrezet et al., 2012).

To explore the recurrence of these variants in the Danish population, we examined if they were included in The Danish Cystic Kidney Disease Registry. Four of ten variants observed in two or more families in the present study were registered in 1–4 families in the registry. We found none of the variants described as recurrent in the literature, see Table 4.

Recurrent variants may be founder variants or identical variants that have arisen independently. The latter are often indels generated by non-homologous recombination or single nucleotide substitutions in CpG dinucleotides (i.e., transitions of C to T or G to A). Indels are frequently generated when recombination occurs between two similar but non-identical sites (Chen et al., 2010). One variant in *PKD2* (c.(2392_2664)_(2861_?)del) was likely generated by non-homologous recombination, and four variants were located at CpG sites: c.6643C > T and c.8293C > T in *PKD1*, and c.1521C > T and c.2614C > T in *PKD2*. These variants were observed in three, two, three, two and five unrelated families, respectively, (Table 4). These five variants may have arisen independently in each family, although it cannot be excluded that some of them might be founder variants, especially the *PKD2* variant observed in five unrelated families.

Five variants that were neither indels nor located at CpG sites remained: two nontruncating variants located in *PKD1* were each observed in two families, and three located in *PKD2* were observed in six, eight and nine families, respectively. The mutation rate in *PKD1* and *PKD2* is thought to be similar to the mutation rate in other large genes associated with autosomal dominant disorders (Rossetti et al., 2001). Although no definite conclusions have been reached regarding how to

predict mutational hotspots, mutations are more frequent in CpG sites and where similar sites increase the risk of non-homologous recombination. Hence, it appears unlikely that the same variants not related to non-homologous recombination or located within CpG sites have arisen independently in up to nine families. Thus, at least some of these variants most likely originated in a common ancestor, i.e. being founder variants.

Similarly, the majority of variants found in more than one family in an Italian cohort (Carrera et al., 2016) and the TGESP cohort (Hwang et al., 2016) were *PKD2* or non-truncating *PKD1* variants. Like our study, these studies also did not recruit patients solely diagnosed by ultrasonic criteria. Truncating *PKD1* variants are associated with a more severe phenotype than variants in *PKD2* and non-truncating *PKD1* variants (Hwang et al., 2016). Patients with truncating *PKD1* variants may therefore have reduced survival and reproductive fitness (Rossetti et al., 2001), which may cause recurrent truncating *PKD1* variants to be underrepresented. Therefore, variants associated with a mild phenotype are more likely to be transmitted through multiple generations, resulting in a high number of seemingly unrelated families harbouring the same variant. In the CRISP (Rossetti et al., 2007) and Genkyst cohorts (Audrezet et al., 2012), the majority of recurrent variants were truncating *PKD1* variants. However, in these cohorts, patients are diagnosed solely by Pei's criteria, decreasing the probability that patients with a mild phenotype are included. This implies that both the frequency of recurrent variants associated with a mild phenotype and the number of families harbouring such variants may be higher than previously reported. Supporting this, the prevalence of ADPKD reported in many studies is lower than the theoretical prevalence reported by Dalgaard (1957) and others (Solazzo et al., 2018).

Conclusion: A pathogenic or possibly pathogenic variant was identified in 87% of the Danish families expected to suffer from ADPKD; 79% and 21% of the variants were identified in *PKD1* and *PKD2*, respectively. 94/112 variants were observed once, and 41 of these were novel. Ten variants in *PKD2* or non-truncating variants in *PKD1* were observed in multiple families. These types of variants often cause a mild phenotype compared to truncating *PKD1* variants. Among the recurrent variants, at least five were most likely founder variants. Founder variants causing a mild phenotype may be more common than previously reported, and the prevalence of ADPKD may be underestimated.

Author statement

Marlene L. Nielsen: Conceptualization, Formal analysis, Investigation, Writing – original draft, Writing – review & editing, Project administration, Funding acquisition; Dorte L. Lildballe: Conceptualization, Investigation, Writing – original draft, Writing – review & editing, Supervision, Funding acquisition; Maria Rasmussen: Conceptualization, Writing – original draft, Writing – review & editing, Supervision, Funding acquisition; Anders Bojesen: Conceptualization, Resources, Writing – review & editing; Henrik Birn: Writing – review & editing, Funding acquisition. Lone Sunde: Conceptualization, Investigation, Writing – original draft, Writing – review & editing, Supervision, Funding acquisition.

Funding

This work was supported by Vanførefonden, Cand. Jur. Torkil Steenbecks Grant, Independent order of Odd Fellows nr. 73 Svend Fælding, Karen Elise Jensen, Danish Kidney Association, Danish Society of Nephrology, and Central Denmark Region.

Declaration of competing interest

Dr. Nielsen reports grants from: Vanførefonden, Cand. Jur. Torkil Steenbecks Grant, Independent order of Odd Fellows nr. 73 Svend Fælding, Karen Elise Jensen, Danish Kidney Association, Danish Society of Nephrology, and Central Denmark Region during the conduct of the study. None of the funders played a role in the collection, analysis and interpretation of data; in the writing of the report; or in the decision to submit the article for publication.

The results presented in this paper have not been published previously in whole or part, except in abstract format.

Acknowledgements

The authors take this opportunity to extend their gratitude to the Department of Clinical Genetics, Aarhus University Hospital, for their collaboration in providing the data for this study.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejmg.2021.104183.

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